HYDROSTATIC PRESSURE EFFECTS UPON THE SPINDLE FIGURE AND CHROMOSOME MOVEMENT. II. EXPERIMENTS ON THE MEIOTIC DIVISIONS OF TRADESCANTIA POLLEN MOTHER CELLS

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Introduction

Hydrostatic pressure increments are known to reduce progressively the rigidity of plasmagels and the viscosity of plasmasols. Eventually complete solation results. Marsland (1939 and 1942) has been able to formulate what appears to be a general quantitative law on the basis of a considerable volume of work with very diverse material. He has found that with each increment of 1,000 lbs./in.^{2} hydrostatic pressure, the relative rigidity or viscosity decreased to 76 per cent of the initial value. This applied no matter whether the cytoplasm of amoebae, *Arbacia* eggs, or *Elodea* was being studied. Furthermore, these direct effects have always proved very rapidly reversible when the pressure was released. The subsequent pattern of cell events, however, has sometimes been found to have been changed by new reorganization patterns (cf., Pease, 1940, 1941).

In the first paper of this series (Pease, 1941), experiments were reported in which advantage was taken of these known effects of hydrostatic pressure to study the first cleavage division spindle apparatus in *Urechis* eggs. The material was not well suited for this sort of work, and some interpretations were open to question. However, the following facts were clear and significant. 1) Pressure could so affect the cell that no trace of the spindle figure appeared in the fixed preparations, and presumably the spindle had been completely liquified. 2) The pressures destroying the spindle blocked all anaphase movement. 3) The chromosomes aggregated in clumps (originally thought to be vesicles) under lower pressures than were required to block anaphase movement. 4) Numerous cytasters appeared in material given a brief recovery period before fixation. 5) Peculiar "half-spindles" developed *de novo* within cytasters whenever the latter came in contact with nuclear material. 6) By their very nature, the half-spindles lacked "continuous fibers" since only one pole was involved, and also there were no "interzonal fibers." 7) Yet there was ample evidence that such half-spindles were functional in moving chromosomes, and even recently-formed nuclei with membranes were at least deformed, and probably moved, by them. The role of cytoplasmic components in the spindle was stressed (perhaps unduly), and the role of the "traction fibers" minimized (perhaps incorrectly as will be seen later).

To find out whether or not nuclear gels behaved in the same manner as cytoplasmic gels when hydrostatic pressures were applied, the extraordinary equational meiotic division in *Steatococcus* spermatocytes has been studied in unpublished work by the author. In these cells the spindle is formed inside the nuclear membrane,

and the anaphase movement nearly completed, before the nuclear membrane disintegrates. In this case, there can be no question but that the whole spindle apparatus is of nuclear derivation. It was found that sufficiently high pressures destroyed it by liquefaction, and anaphase movement was blocked. The spindle re-formed once more when the pressure was removed and the cells allowed a short recovery period. Thus the physiological action of hydrostatic pressure appears to be qualitatively identical in gels of nuclear and cytoplasmic origin.

For the present work, *Tradescentia* pollen mother cells (PMC) were selected as material for several reasons. The spindle is characterized by relatively enormous "traction fibers" going to the poles from comparatively large and easily visible kinetochores. The cells have the advantage of a small number of chromosomes which are relatively large. The only important disadvantages are the impossibility of getting controls which necessarily divide at the same time as the experimental material, and the extreme difficulties (which proved insuperable with pressure techniques) of actually observing the divisions *in vivo* (cf., Shimakura, 1934).

The material was collected and prepared at Stanford University, and the author is indebted to Dr. Reed Rollins of that institution's botany department for technical advice on handling procedures and for the plants which were used. The material was studied mostly at Columbia University before the war interrupted the work. Dr. F. Schrader, Dr. S. Hughes-Schrader, and Dr. H. Ris followed its course with interest, enthusiasm, and valuable suggestions. Dr. C. W. Metz of the University of Pennsylvania also contributed excellent comments on an early draft of the manuscript.

Material and Methods

The half dozen *Tradescantia paludosa* plants used in these experiments possessed six pairs of chromosomes. They were derived from a common stock. The anthers were prepared by separating the connective which joins the two lobes. One lobe was then fixed as a control just at the time of pressure application to the other lobe. The bisection of the anthers with a small lance could be accomplished easily without rupturing the anther lobe walls. The lobes were handled and finally mounted in a 7.4 gm./100 ml. saccharose (Merck, C. P.) solution which Shimakura (1934) has found to be isotonic with *Tradescantia* pollen mother cells.

The pressure bomb used in these experiments held a half dram homeopathic vial, and was so designed that it could be opened very rapidly. After filling with sugar solution and a few anther lobes, the vial was sealed with "Parafilm" wax sheet held in place with a rubber band. The experimental material was always kept under the desired hydrostatic pressure for a one hour period. In a few experiments the material was fixed 30 minutes after the release of pressure which allowed time for some recovery. But in most of the experiments, the pressure was released, the bomb opened, and the fixative added within one minute. Preliminary experiments had shown that there was no appreciable reorganization within that short time limit.

Experiments were performed using 1,000 lb. pressure increments from 1,000 to 6,000 lbs./in.^{2}, and with 8,000, 10,000, and 15,000 lbs./in.^{2} Control experiments were performed giving identical treatment, but at atmospheric pressure, and at the relatively low pressure of 150 lbs./in.^{2}

Bouin's fixative, to which 3 per cent urea was added, was used throughout. For study, eight micra sections were prepared, and stained by Heidenhain's hema-

toxylin method. Both mordanting and staining were prolonged (never less than 5 hours each), and the sections were destained in saturated picric acid in such a fashion that considerable stain remained in the cytoplasm. There was a good deal of shrinkage, but the cytoplasmic differentiation (particularly of the spindle) was good.

Results

Effects upon the first division spindle

The first division spindle was particularly sensitive to a critical hydrostatic pressure that was found to be between 4,000 and 5,000 lbs./in.^{2} Even after 4,000 lbs. had been applied, the spindle figures looked essentially normal. There was no reduction in the length or diameter of "traction fibers" (compare Fig. 28 with Figs. 25 and 26). However, many of the "continuous fibers" had apparently been lost for the net effect was a more diffuse looking spindle mass with fewer and less conspicuous continuous fibers. The abnormalities of chromosome movement under even the lower pressures prevented any adequate study of "interzonal connections," but occasional examples that looked normal have been found after 4,000 lbs./in.^{2}

In striking contrast were the results after 5,000 lbs. had been applied. The traction fibers were then reduced in length and in diameter so that they appeared as delicate structures (Fig. 30). Small numbers of faint and very thin continuous fibers were usually visible, although not always. Ordinarily 6,000 lbs. pressure obliterated the spindle completely, but in a small fraction of the cells a fine residual fiber structure remained visible. Figure 31 is a photograph of the heaviest and most extensive fibers which have been observed in material fixed after an exposure to this pressure. It must be emphasized that this is an entirely atypical cell. No sign of continuous fibers has been seen after exposures to 8,000 lbs., and it was the very rare cell which showed indications of traction fibers. When visible, as in Figure 33 (arrows), they were thin and short. No oriented fiber structure of any sort was ever observed after exposures to 10,000 or 15,000 lbs./in.^{2}

In summary, it can be said that the first division spindle looked essentially normal after treatments with 4,000 lbs./in.^{2} pressure, but was profoundly affected by 5,000 lbs. This demarkation was really very sharp!

Effects upon the second division spindle

The spindle of the second meiotic division was considerably more resistant to hydrostatic pressure than that of the first division. The spindles appeared nearly normal after 4,000 lbs./in.^{2} pressure, and after 6,000 lbs. the spindles of some cells did not seem to be greatly affected. After 6,000 lbs. pressure there was a considerable individual variability in different cells, even within the same anther lobe. The best spindles were somewhat fainter than normal, and the fibers seemed generally thinner, but they sometimes extended from one pole to the other. After 8,000 lbs. pressure there were occasionally evidences of traction and continuous fibers, although they were always thin and faint if present. No fiber structure was ever visible after pressures of 10,000 lbs. or more.

It thus appears that the second division spindle withstood nearly 2,000 lbs./in.^{2} more pressure than the spindle of the first division. It will appear later that the pressure required to block anaphase movement was similarly proportional.

It may also be noted here that there was a little evidence that the spindles of the somatic cells in the connective were even more resistant to pressure, and were not entirely destroyed unless pressures in excess of 8,000 lbs. were applied.

Effects upon the chromosomes—fusion

Increasing hydrostatic pressures made the chromosomes progressively more "sticky" and "soft." Chromosomes tended to aggregate in fused masses. In Figure 27 a metaphase plate is shown, fixed just after the release of 2,000 lbs. pressure. It will be noted that there are stained "bridges" connecting all of the chromosomes. At this low pressure, the bridges were, on the average, only slightly heavier than comparable bridges which could be found in controls of the proper stage. However, they persisted much longer than normally, well into the anaphase stages.

When pressures of 3,000 lbs. or more were applied, the inter-chromosomal bridges tended to become much thicker, and entirely out of the range of normal variation. Figure 32 shows such connections in a cell fixed just after the release of 6,000 lbs. pressure. With progressively higher pressures, there was an increasing tendency for the fusion of chromosomes into a single mass. This can be seen in Figures 33 and 34. The extreme condition was reached at 15,000 lbs./in.^{2} when it was nearly always quite impossible to recognize individual chromosomes. This is well shown in Figure 36.

It must be emphasized that the preceding description and the photographs are typical of cells to which the pressure was applied in late metaphase stages. When the pressure was applied to early metaphases, the chromosomes showed a much greater degree of fusion for corresponding pressures. Of considerable importance must have been the proximity of chromosomes, and probably also the initial presence of thin connections. The existence of some movement in the low pressure range may have aided the process.

Not only were metaphase chromosomes fused together by treatment with hydrostatic pressures, but a comparable effect was observed with late diakinesis chromosomes before the nuclear membrane broke down. Here the chromosomes are apparently normally kept separate from one another by gel structure within the nucleus, for nucleoplasm strands showed clearly enough in fixed preparations. These strands continued to be visible until pressures of 6,000 or 8,000 lbs./in.^{2} were applied. As long as they were present the chromosomes kept apart and did not fuse. After the higher pressures the strands were no longer visible, and the chromosomes were all in a single clump together. But, as with the metaphase chromosomes, the individual chromosomes did not lose their visible identity until pressures of 15,000 lbs. were applied.

At metaphase, the chromosomes were not only found fused laterally in the plane of the equatorial plate, but the homologous chromosomes were also fused together so that their separation was greatly complicated. This was very obvious when first division anaphases fixed just after the release of 3,000 or 4,000 lbs. pressure were studied. Practically every cell showed evidences of fusion with bridges that were often long and massive (cf., Figs. 1–12). Such bridges always stained just as the chromosome proper with hematoxylin (Fig. 39), and the larger ones, at least, were stained by the Feulgen reaction. These bridges were frequently between homologous chromosomes, but also commonly involved lateral fusion with non-homologous chromosomes.

Even more massive bridges were found in the second meiotic division material subjected to the higher pressures which still allowed a good spindle to exist. Then, after 6,000 lbs./in.^{2} pressure, most or all of the chromosomes were frequently so fused together that they nearly lost their visible identity. However, the mass of chromosomes often would be strung out from one end of the cell to the other (Fig. 15).

It should be noted that the chromosomes of somatic cells showed the same type of fusion. These have occasionally been seen in the tissue of the connective, and Figure 41 shows one bridge out of a total of three present in such a cell fixed just after the release of 4,000 lbs. pressure.

Effects upon the chromosomes—rounding

It should be emphasized that all of the fusion bridges between chromosomes had rounded outlines. This shows well in Figures 27 and 32, and suggests a considerable plasticity.

In addition, the chromosomes as a whole tended to round up under the higher pressures. This was most obvious in the second division chromatids which were V-shaped with relatively long and thin arms. After 3,000 lbs. pressure there was very little noticeable change in shape even though there might be some fusion (Fig. 13). However, after 4,000 lbs. there was a striking alteration. The chromatids were then decidedly thickened and shortened (Fig. 14). This tendency became more pronounced with increased pressures (Fig. 15, 6,000 lbs.).

The short and thick chromosomes of the first meiotic division were not as suited for study, but the same tendency was obviously present. Particularly after 10,000 lbs., when the identity of individual chromosomes could still be seen, they were decidedly shortened and rounded except at the kinetochore region (Fig. 34).

Effects upon the chromosomes—the spindle attachment region

The first meiotic division material gave the impression that 1,000–3,000 lbs./in.^{2} pressure allowed a greater extension of the attachment region of the chromosomes than was normal (compare Fig. 26 with 25). More particularly, this region of some chromosomes was extended far beyond what could be found in the controls. The attachment region gave the impression of being unduly short in the material exposed to 4,000 lbs. pressure. An attempt to measure statistical samples was decided upon.

In Table I the mean extensions of the attachment regions of first division chromosomes are given for pressures up to 4,000 lbs./in.^{2} There were, of course, real difficulties in measuring such small distances, but errors should have cancelled out in the averages. While no great reliance should be placed on the absolute values, they certainly indicate the general trend.

The measurements were made with a filar micrometer. In each group, 50 measurements were made at random, excepting that only cells in anaphase were selected, and individual chromosomes that had not yet separated and left the metaphase plate were measured. The micrometer hair was moved up to a chromosome until it just touched the distal tip of the kinetochore (indicated by the arrows in Figs. 25 and 26), and a reading made. Then the hair was swung across the field, and moved back in the other direction until the hair just touched the base of the attachment

stalk which was ordinarily rather well defined from the body of the chromosome by its relative translucency. Then a second reading was made. The difference measured the length of the stalk plus the width of the hair in the micrometer. The hair width was measured in the same way in relation to a fixed point, and this value was subtracted from all of the measurements. The figures were then converted to micra. The control measurements actually used for comparison were combined from data upon the control anther lobes of the 1,000 and 3,000 lb. experimental material, and a control anther which was left mounted in the bomb for one hour before fixation, but without pressure.

It is to be concluded that the mean length of the attachment stalk was definitely increased by pressures from 1,000 to 3,000 lbs./in.^{2}, and it has also been found that there is no overlap in the extreme extensions between control cells and experimental cells exposed to this pressure range. With 4,000 lbs. pressure the mean extension was significantly less than in the controls, and the greatest extensions found after this treatment did not even approach the maxima found in the controls.

TABLE I

Pounds pressure	Mean extension in micra	Percentage increase in length	Percentage overlap with control mean
control	0.85		
1,000	1.4	59.4	2
2,000	1.2	38.6	6
3,000	1.2	42.9	4
4,000	0.68	−19.9	22

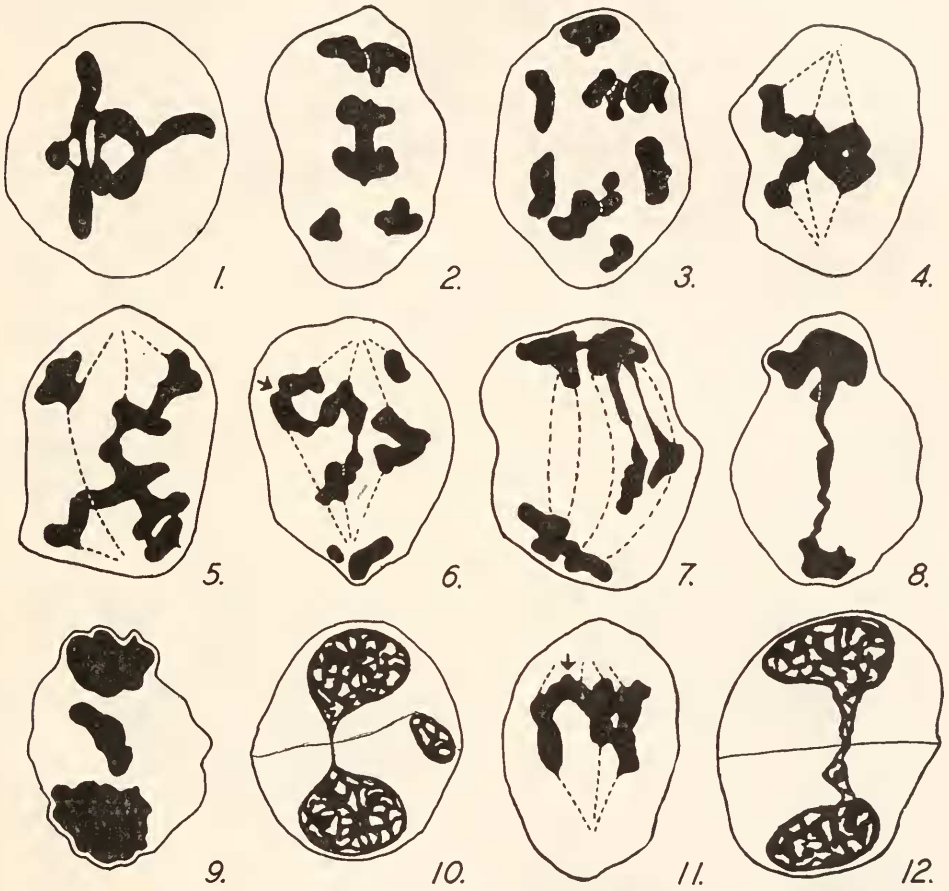
The distance between the tip of the kinetochore and the base of its stalk is given in the second column. In the experimental series, 50 measurements were made at random, excepting only that early anaphase cells were selected. The control average, however, is a combined average of three sets of measurements upon different material. The mean percentage increases in length are based upon figures carried to the third decimal place. The last column gives the percentage of measurements which overlapped the mean of the control.

Effects upon the chromosomes—chromonemata

We have already seen that late prophase and metaphase chromosomes fused together and rounded up under the influence of hydrostatic pressure. This, however, only applied to condensed chromosomes. Uncondensed early prophase chromosomes did not seem to be affected by even the highest pressures employed. This agrees with the findings of Pease and Regnery (1941) who were unable to detect any effect of 15,000 lbs./in.^{2} pressure upon *Drosophila* salivary chromosomes which are similarly uncondensed. It must be admitted that no detailed study has been made of the early prophase chromosomes. While there was certainly no general clumping, it is possible that very local fusions could have been overlooked, but there was no indication of shortening or thickening.

An "accidental experiment" gave further information, and additional reason for believing that the chromonemata were not affected by hydrostatic pressure. An anther lobe which had been pricked was exposed to 15,000 lbs./in.^{2} pressure for one hour and was then rapidly fixed in the usual fashion. The surrounding sugar solu-

tion had entered the anther, and apparently was somewhat hypertonic. All of the cells were slightly plasmolized and had more or less swollen chromosomes. In one small section of the anther, conditions were such that the spiral structure was visible. Figures 37*a* and *b* are photographs of one of these early anaphase cells, and it is obvious that the spiral structure was unaffected. Oddly enough there was no tendency for the chromosomes to fuse under these circumstances.



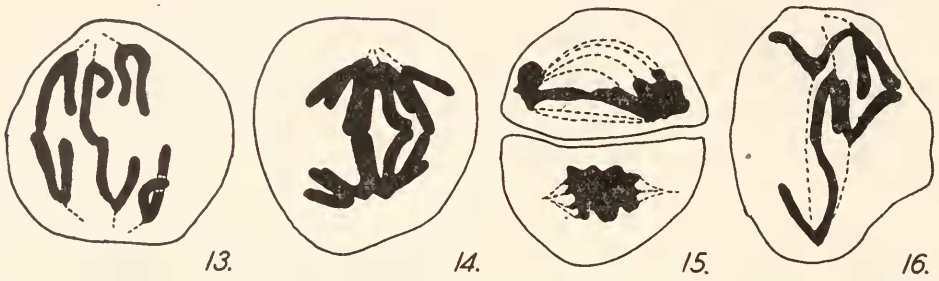
First division cells. Figures 1–10 are of sections from material which was fixed just after the release of 4,000 lbs./in.^{2} pressure. Figures 11 and 12 are of sections fixed just after the release of 3,000 lbs. pressure. The broken lines represent traction fibers except in Figure 7 where they represent the pathways of "continuous fibers." All of the chromosomes visible were not necessarily included.

Abnormalities of chromosome movement under pressure

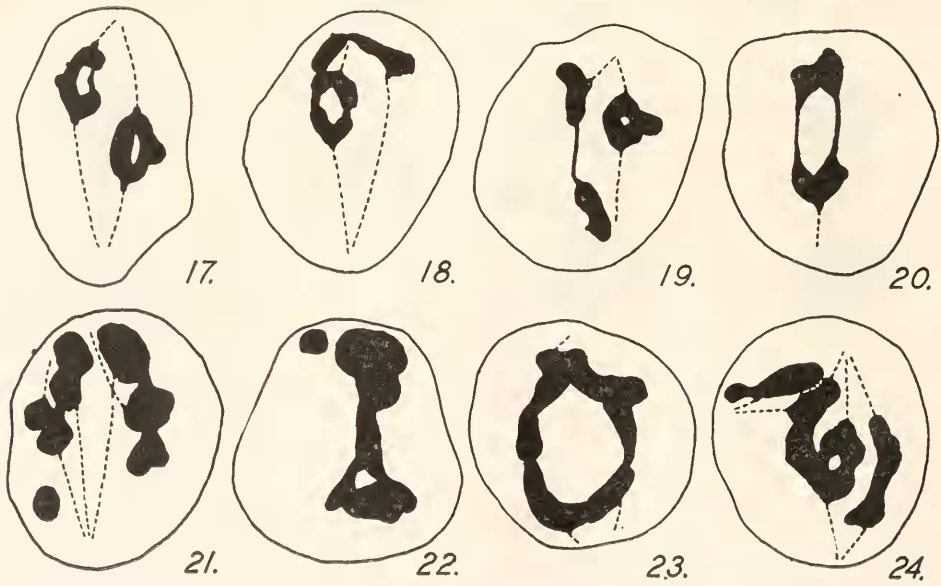
Because of the fusion of metaphase chromosomes, even by relatively low pressures, their ultimate distribution to the two spindle poles was usually very abnormal whenever anaphase movement took place during the pressure treatment. The particular pattern which resulted apparently depended upon the balance between ana-

phase forces and the local resistances of whatever fused bridges happened to be present. Greater or lesser fusions might occur between homologous chromosomes and, laterally, between non-homologous chromosomes. Almost any conceivable variation in the resulting pattern could be found in all degrees. Some of the more interesting variations which have been seen are included in Figures 1–15, which are also perfectly typical of material exposed to 3,000 or 4,000 lbs. pressure.

Homologous chromosomes might be so extensively fused that separation could not occur. Such pairs of chromosomes, fused as in Figure 2 in the metaphase plate



Second division cells. Figure 13 is from material fixed just after the release of 3,000 lbs./in.^{2} pressure; Figure 14, after 4,000 lbs. pressure; and Figure 15, after 6,000 lbs. pressure. Figure 16 is from recovery material, fixed 30 minutes after the release of 10,000 lbs. pressure. The broken lines indicate traction fibers except in the upper cell of Figure 15 in which they indicate the pathways of the "continuous fibers." Not all visible chromatids were necessarily included.



Figures 17–24 are all from first division recovery material which was fixed 30 minutes after the release of 10,000 lbs./in.^{2} pressure. The broken lines indicate traction fibers. Not all visible chromosomes were included except in the last three figures.

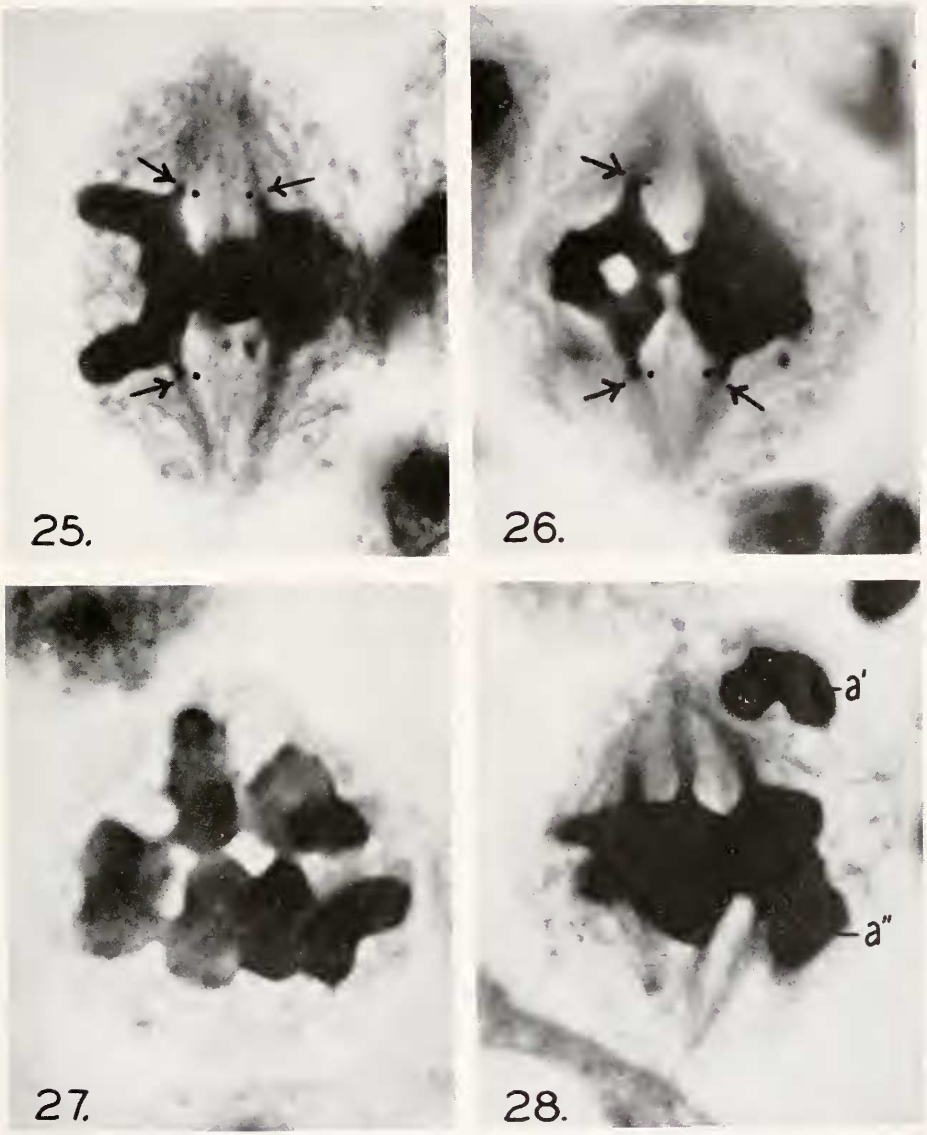


Figure 25 is a first division early anaphase control exposed in the bomb for an hour (but without pressure) before fixing. Figure 26 is of a cell fixed just after the release of 2,000 lbs./in.^{2} pressure. Figure 27 is a metaphase plate of the same material. Figure 28 is of a cell fixed just after the release of 4,000 lbs. pressure, and note the anaphase separation of the homologous chromosomes *a'* and *a''*. The small arrows indicate the distal ends of the kinetochores. The magnification of these and the following photographs is approximately × 3,000.

region, would presumably have remained there, and eventually formed micronuclei (Figs. 9 and 10).

Even though there was no lateral fusion with other chromosomes, there might be slight differences in the forces directed towards the two poles, or possibly in the strength of the traction fibers going to opposite poles. An extensively fused pair of chromosomes might then go as a unit to one pole (Fig. 5). Then there would always be an abnormally long, but otherwise normal looking traction fiber (with full thickness) going most of the way across the cell to the other pole.

Figures 4 and 5 show very extensive lateral fusion between non-homologous chromosomes. Such anaphase cells would probably have given rise to extensive bridges in telophase, and between daughter nuclei, such as are shown in Figures 8, 10, and 12.

In Figure 6 the lower member of a pair of homologous chromosomes, indicated by an arow, was laterally fused with a non-homologous chromosome going to the upper pole. Seemingly it was being carried to that pole in spite of its traction fiber to the other pole.

We have already spoken of the massive bridges which characterized the second meiotic division material exposed to 6,000 lbs. pressure, and which often involved all of the chromatids (Fig. 15). There was less fusion with lower pressures, and the abnormalities more nearly resembled what has just been described for the first division.

The critical pressure blocking anaphase movement

The best evidence for chromosome movement under pressure is certainly the presence of extensive bridging. The author sees no rational way of accounting for the bridges other than to suppose that anaphase movement occurred after the chromosomes established fusions in the metaphase plate and then pulled out the bridging connections.

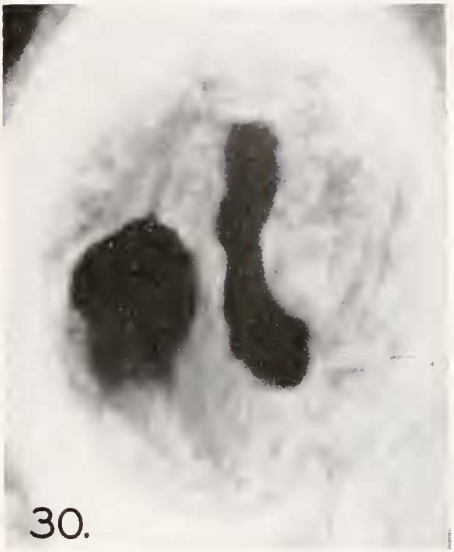
With this as a criterion of movement, it is possible to state that anaphase movement continued at 4,000 lbs./in.^{2} hydrostatic pressure in the first meiotic division, but was blocked by 5,000 lbs. pressure. No extended bridge has been seen in any cell of this division exposed to 5,000 or more pounds pressure. Nor were there ever signs of asynchrony, or of directionally atypical movements.

It must also be emphasized that abnormal division resulting from fusion characterized practically *every* anaphase cell exposed to 4,000 lbs. pressure. It was also extremely common after 3,000 lb. treatments. Similar abnormalities appeared on a lesser scale after 1,000 or 2,000 lbs., but then the separation was more frequently fairly normal, and characterized only by loss of division synchrony.

In the second meiotic division very abnormal anaphase movement involving massive fusions took place in some cells exposed to 6,000 lbs./in.^{2} pressure (Fig. 15), but none was possible at 8,000 lbs.

Bridging has been found even after 8,000 lbs. pressure in the somatic cells of the connective. Figure 42 is from a somatic cell forming daughter nuclei at this pressure, and two out of a total of five bridges are visible in the plane of the photograph.

In the meiotic divisions, at least, the presence of a good visible spindle was correlated with anaphase movement. When the spindle was obviously considerably af-



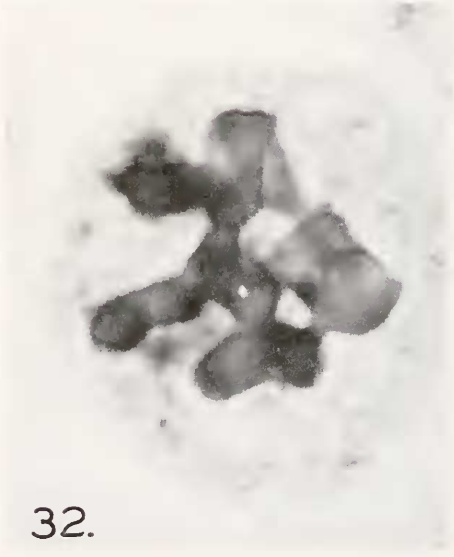


Figure 29 is a late anaphase cell from the same material as Figure 28 (exposed to 4,000 lbs./in.^{2} pressure). Figure 30 is a cell fixed just after the release of 5,000 lbs. pressure. Figures 31 and 32 are from material fixed just after the release of 6,000 lbs. pressure.

fected there were no longer evidences of anaphase movement. This was also probably true of the somatic cells, but they have not been carefully studied. It is clear that movement is most sensitive to hydrostatic pressure during the first meiotic division, withstands nearly 2,000 lbs. more pressure in the second division, and seemingly about 2,000 lbs. more in the somatic cells. This, in turn, appears due to different characteristics of the spindle gels, rather than being due to differential pressure effects upon the chromosomes. For in the first and second meiotic divisions, and probably also in the somatic divisions, the chromosomes seemed affected equally by equal pressures.

Spindle recovery after pressure release

At the time of making these experiments the importance of the recovery stages was largely unsuspected, and relatively little material was gathered. But after one hour exposures to 10,000 and 15,000 lbs./in.^{2} pressures, some experimental material was removed from the bomb and given a 30 minute recovery period before fixing. Many of these cells showed excellent spindles with massive traction fibers (Fig. 38).

Of particular interest is the fact that the traction fibers of these recovery spindles were *de novo* formations. Conclusive evidence of this was afforded by paired homologous chromosomes (still fused as a result of the pressure treatment) which formed traction fibers from both kinetochores that went to the same pole. Figure 39 is a photograph of such a condition. Figure 40*a* is a drawing of another example. Figure 40*b* seems further complicated for apparently one traction fiber had to curve around a blocking chromosome before its direction to the "wrong" pole could become definitive. In Figure 40*c* each traction fiber can probably be considered as having gone to the "wrong" pole so that the original polarity of each chromosome was entirely reversed.

Figures such as those described in the last paragraph were not rare, although out of the ordinary. They were never seen in the controls, nor is the author aware of similar accounts in the literature.

Most commonly the spindle appeared to re-form nearly along its original axis if it is assumed that the metaphase plate was not displaced, and remained as an index of that polarity. The pattern thus usually seemed very nearly normal. However, the long axis of the new spindle was sometimes very oblique to the plate, and presumably to the original spindle axis. In extreme cases a 90° shift was indicated.

Also, not infrequently multipolar spindles were found which were very rare in the control material. Three-pole spindles such as Figure 24 were fairly common, and a few four-pole spindles have been seen. All possible variants were seen with equal or very unequal poles, spaced equidistant from one another, or barely separated.

These several lines of evidence all imply that the spindle was re-formed *de novo*, and was not rebuilt upon residual structure which had survived the pressure treatment and persisted to give a framework. New patterns appeared, and whatever molecules were involved, they were at least rearranged.

The development of the recovery spindle

One can select a series of cells which apparently show the different steps of spindle re-formation after the release of pressure. In some cells fiber structure con-

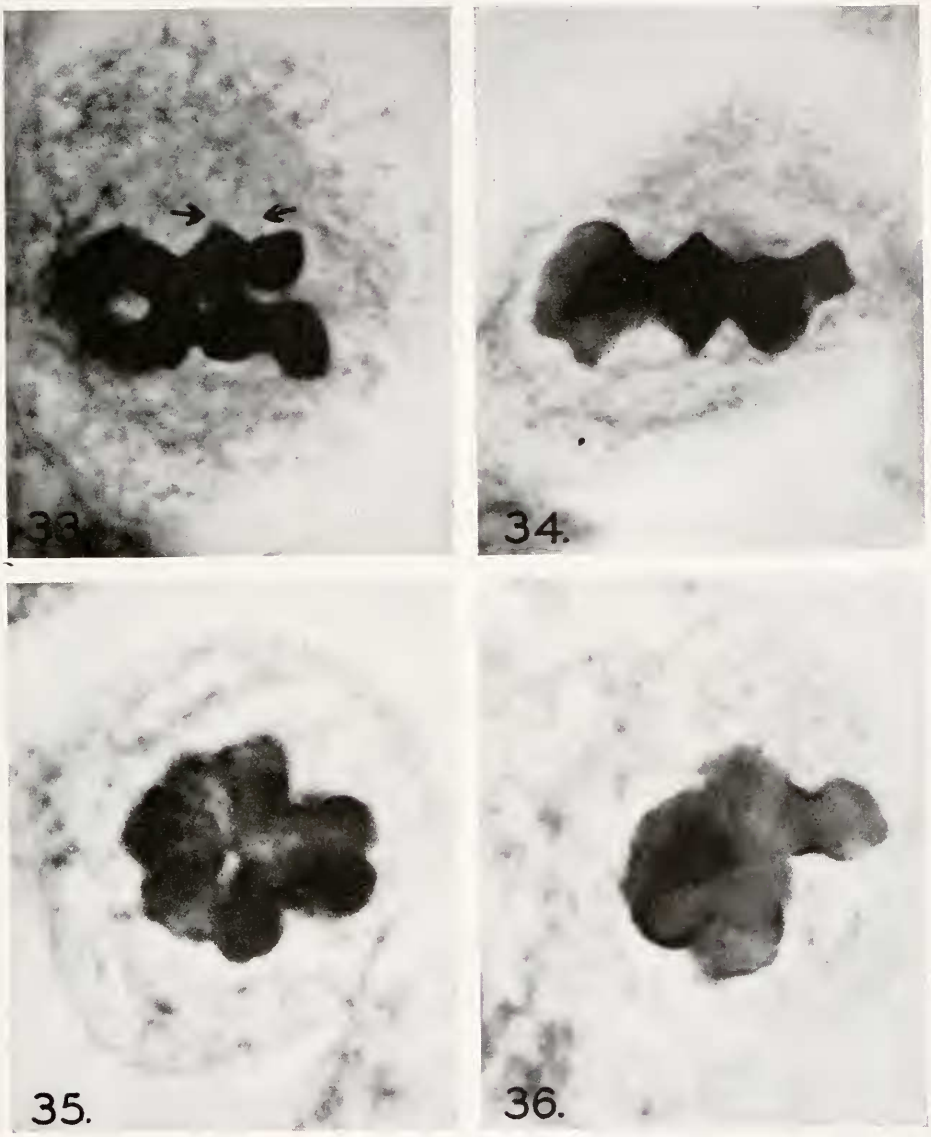


Figure 33 is of a cell fixed just after the release of 8,000 lbs./in.^{2} pressure (the arrows indicate very faintly visible traction fibers), and Figure 34 after 10,000 lbs. pressure. Figure 35 is of a cell fixed just after the release of 15,000 lbs. pressure, and the orientation is thought to be in the plane of the original spindle axis. Figure 36 is from the same material, but sectioned in the plane of the metaphase plate.

sisted of thin fibrils tangled around the clumped chromosomes of the equatorial plate, and without any polar orientation. The fiber direction was roughly circumferential to the enclosed mass of chromosomes (as in a cocoon, Fig. 44). This could be regarded as the first recovery stage.

Many cells showed polarized fibers as in Figure 45*a*. The section of Figure 45 is oblique to the spindle axis. The focus of Figure 45*a* is tangent to the slant height of the cone which makes up one-half of the entire spindle (the "surface" of the spindle, so to say). The visible fibers are the continuous fibers of the new spindle. Figure 45*b* is a lower focus of the same cell. It should be observed that there are no continuous fibers in the center of the cone. Instead, there are only slight indications of traction fibers. The continuous fibers were thus largely peripheral, but the extensive lateral fusion of the chromosomes to make a practically solid metaphase plate probably had much to do with this morphological pattern which was typical of recovery material.

Traction fibers were not seen in cells without polarized continuous fibers. But when the latter had formed, traction fibers could usually be found. In some cells they would be thin and short, in others longer and more massive. Thus the traction fibers appeared to "grow" outward directly away from the kinetochore region, and full thickness was not achieved until they practically reached the poles.

It was possible to find many minor irregularities in the developmental pattern of traction fibers. These resulted whenever the kinetochore pointed in some other direction than directly towards a pole. A graded series could be found, the extreme examples being when kinetochores pointed more or less to "wrong" poles. Invariably the base of the traction fiber extended directly away from the kinetochore, and it did not bend towards a pole until it became associated with continuous fibers. The bend would then be towards the pole less than 90° away from the initial growth direction even if this happened to be the "wrong" pole. It thus looked as though the growth direction was unimpeded until the traction fiber became associated with continuous fibers, and then the further extension of the traction fiber followed the path of least resistance in the pattern expressed by the continuous fibers. Thus the traction fiber even developed around obstructions as in Figure 40*b*.

The fusion of traction fibers

A very rare situation casts further light on the formation of traction fibers if the interpretation is correct. It was possible to find non-homologous chromosomes in the recovery material which appeared to be bridged across the kinetochore regions. A photograph of such a bridge is shown in Figure 43. These bridges differed from all the other ordinary bridges which have been seen in that they were achromatic. Although they were short, they had exactly the appearance in the fixed and stained preparations that traction fibers had. They certainly gave the impression that they represented fused traction fibers, traction fibers which started to develop from each separate kinetochore in opposite directions, and which grew terminally into each other to fuse end to end.

The author hesitates to emphasize these structures. The material has been thoroughly searched and only two good examples have been seen, plus another which was more questionable because overlying material partially obscured it. There may be good reason for their rarity, for it is obviously an exceptional situation to have two kinetochores pointed directly towards each other. If we accept their

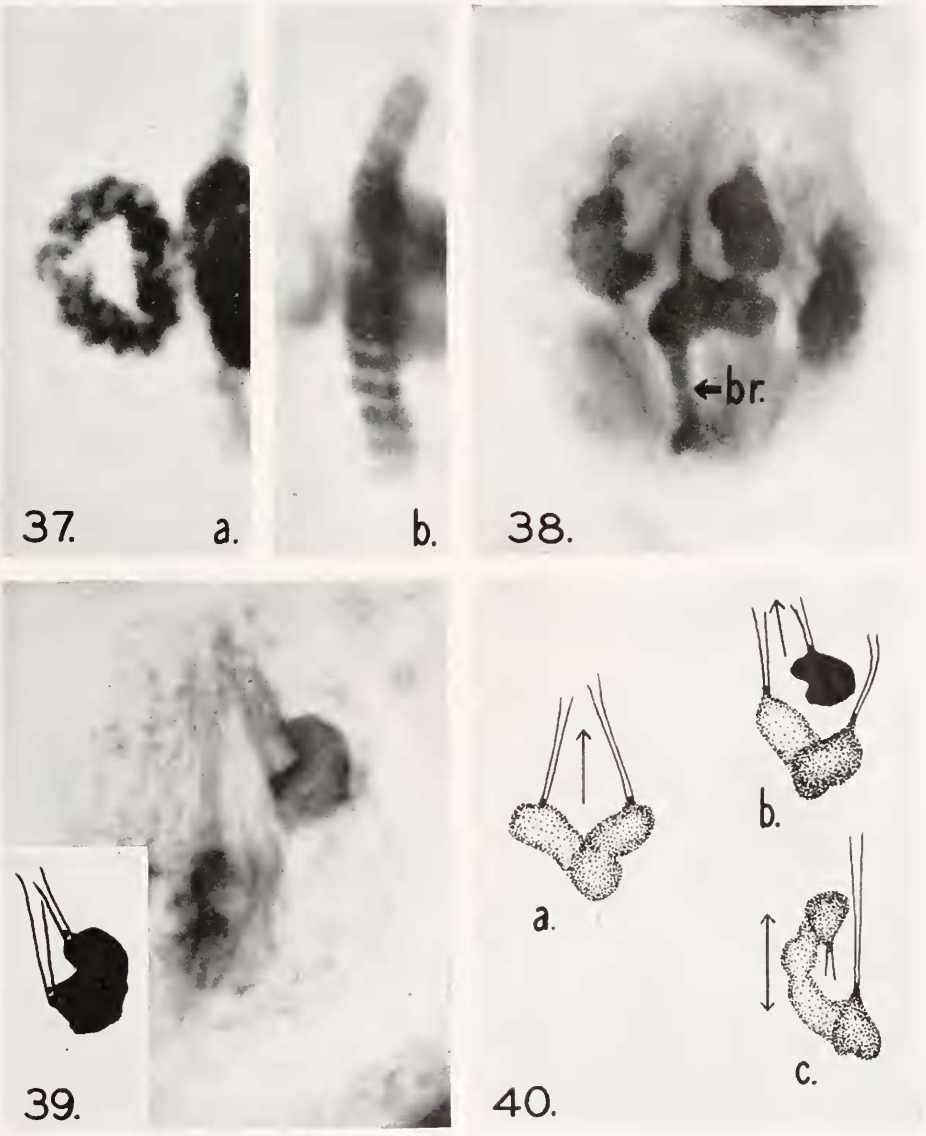


Figure 37 is from a slightly plasmolized cell fixed just after the release of 15,000 lbs./in.^{2} pressure (*a* and *b* are different focal levels). Figures 38–40 are from recovery material fixed 30 minutes after the release of 10,000 lbs. pressure. In Figure 38 note the bridge, *br*. In Figures 39 and 40 *de novo* recovery traction fibers of fused homologous chromosomes go to the "wrong" pole. The direction of a pole is indicated by arrows in Figure 40.

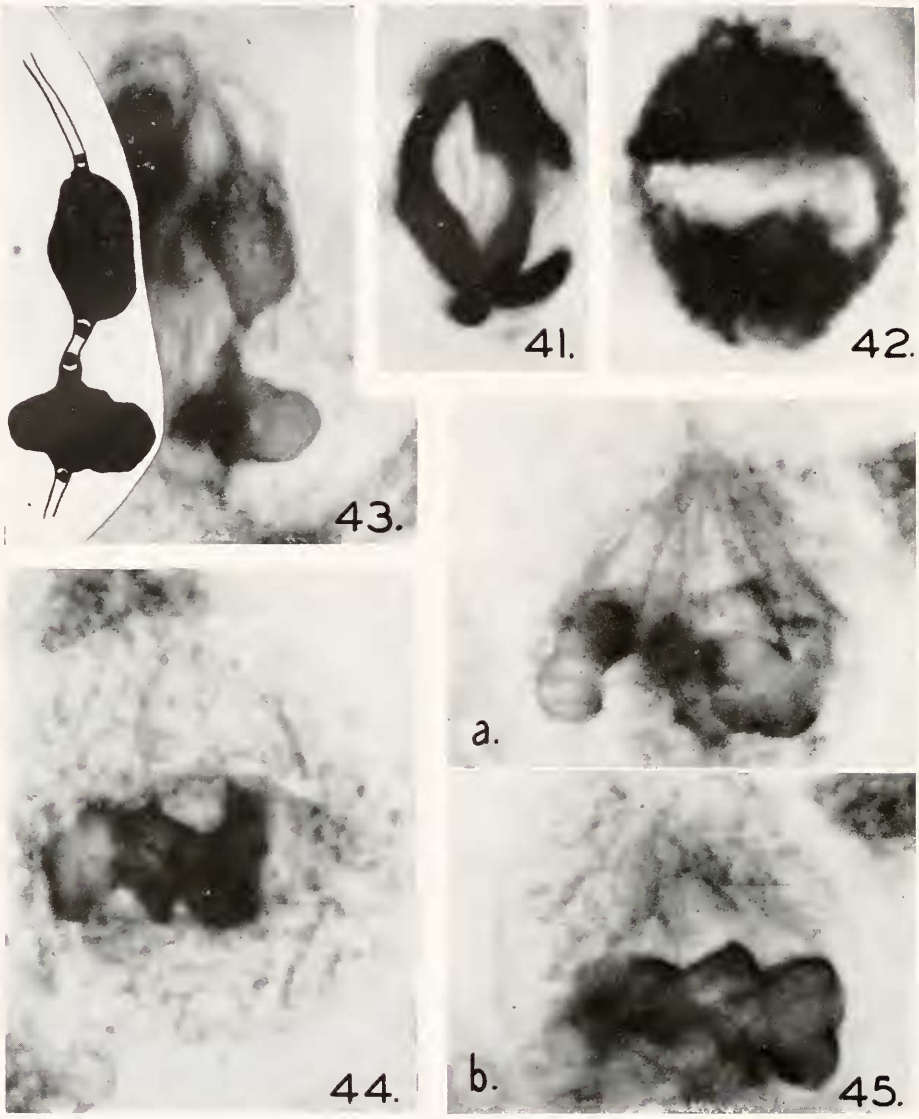


Figure 41 is of a somatic anaphase cell fixed just after the release of 4,000 lbs./in.^{2} pressure. Figure 42 is of a somatic cell forming daughter nuclei, fixed just after the release of 8,000 lbs. pressure. Figure 43 is from recovery material fixed 30 minutes after the release of 10,000 lbs. pressure, and shows achromatic bridging between non-homologous chromosomes (fused traction fibers?). Figure 44 shows an early stage of spindle recovery in material fixed 30 minutes after the release of 10,000 lbs. pressure. Figure 45 is from the same material, but spindle recovery is more advanced (*a* and *b* are different focal levels of the same cell).

reality and the above interpretation, however, the implications are of considerable interest, for it means that developing fibers can mutually terminalize each other. Yet there is no effect as far as lateral growth is concerned, and the fibers thicken as normally. There is simply no growing end left. We can say that fibers extend by terminal additions rather than from the kinetochore, or by elongation from within their length.

Having gone this far, we can make another deduction as to the role of the kinetochore in traction fiber formation. We can regard it as an "organizing center" which initiates linear extension and controls fiber diameter. The linear gowth is self perpetuating once started until the fiber reaches a pole, or is terminalized as above. The fiber thickens by further organization at the kinetochore, and additional linear growth parallel to the initially thin fiber, thus adding enclosing layers. The final fiber has a thickness equal to the diameter of the organizing center. The author reiterates that this hypothesis has a slender experimental basis, and depends upon a correct interpretation of three figures.

Chromosome movement in recovery material

There were obvious indications of chromosome movement in recovery material after the spindles re-formed. The movement was abnormal because of strong and persistent fusion bridges, and in many ways resembled the anaphase movement which occurred under low pressures (3,000 and 4,000 lbs.).

Frequently fused pairs of homologous chromosomes were found going to, or after they had reached, a single pole as in Figures 38 and 17. In such cases one traction fiber extended all the way across the cell to the other pole but seemed to be of normal thickness. This type of movement often seemed to be aided by lateral fusion with non-homologous chromosomes as in Figures 18 and 21. Less frequently the fusion between homologous chromosomes was relatively slight, and there would be a partial separation with the formation of more or less long and thin bridges (Figs. 19, 20, and 38, *br.*). Quite frequently very massive bridges were formed involving most if not all of the chromosomes which would be fused together (Figs. 22 and 23). There were no important differences between first and second meiotic division cells (note Fig. 16).

None of the material was allowed a sufficient recovery period so that daughter nuclei formed in cells which began their anaphase movement after the application of pressure. It can be presumed, however, that many of the cells would form only a single nucleus because of an inability on the part of the chromosomes to separate. Other cells would be expected to form bridged nuclei, and probably multiple micronuclei.[1]

Chromosome structure in recovery material

The persistence of chromosome fusion in the recovery material would seem to suggest just one possibility—that the initial fusion under high pressure must have been due to at least a partial liquefaction of some chromosomal element, and that the fusion bridges then gelled when the pressure was released. In the recovery material the chromosomes were thus stuck together by very viscous bridges. After examining a great deal of material, the author is of the opinion that it is very doubt-

[1] Pease (1941) definitely found this to be the case in *Urechis* eggs.

ful that fused chromosomes were ever able to separate completely before the formation of daughter nuclei. Most commonly there were few signs of any separation, but even in extreme cases, thin and very long bridges persisted as in Figures 19 and 20. The moderately thick bridges, at least, stained with Feulgen.

There is another, and much more puzzling, aspect of chromosome structure which is brought to light by a study of the recovery material. Even after the release of 15,000 lbs./in.^{2} pressure (which resulted in the very complete fusion of the chromosomes as in Figure 36) the chromosomes regained their visible identity and their approximately normal shape. This tendency can be seen (in 10,000 lbs. material) by comparing Figure 38 with Figure 34, but it is best seen by comparing the long chromatids of the second meiotic division (compare Fig. 16 with Figs. 14 and 15). In regaining the normal shape, the fusion areas must necessarily have been reduced in cross-section, and it is likely that some fusion bridges were lost entirely during this change. The effects of this change were best demonstrated by the separation of the second division chromatids in material recovering from 10,000 lbs. pressure. Extensive separation sometimes occurred, thus differing in degree from the first division. Figure 16 gives an indication of typical difficulties which were essentially the same as in the first division.

Absolute pressure and recovery rate

In *Urechis* egg material Pease (1941) found that the rate of recovery was roughly proportional to the absolute pressure which had been applied. In the *Tradescantia* PMC material we can only compare the effects of 10,000 and 15,000 lbs./in.^{2} pressures. Comparison is subjective, but there was not the slightest doubt but that the cells subjected to 10,000 lbs. pressure showed a much greater amount of recovery of the spindle elements in 30 minutes than the cells exposed to 15,000 lbs. showed in the same length of time. Fully developed new spindles were only rarely found in the 15,000 lb. material, but were common in the 10,000 lb. material. In both, however, the chromosomes had regained their visible identity and approximately normal shapes.

Conclusions

A single hypothesis readily accounts for most of the manifold effects of hydrostatic pressure upon spindle, chromosomes, and anaphase movement. This supposes that increasing hydrostatic pressures progressively reduce gel rigidity, with liquefaction as the end result. Conversely, after the release of pressure, conditions return to a state such that gel structures can be re-formed once more. There is, of course, an excellent experimental background for this thesis, particularly in so far as it applies to cytoplasmic systems. This has been indicated in the introduction, and has been outlined at greater length in the first paper of this series (Pease, 1941).

It is, however, unfortunate that this work depends upon an interpretation of fixed material. However, we have every reason for believing that the presence of good fiber structures in such material is a good index of oriented gel structure in life. It is only on that assumption that a comprehensive pattern appears, consistent throughout its details. It is true that whenever we have contributory evidence of liquefaction (such as a block of anaphase movement), we do not find fiber structures in the cytological material. Apparently extensive fiber structures are only

precipitated by fixation agents when molecules are at least organized into an oriented pattern and probably also concentrated in a gel.

Spindle structure and formation

In view of the above considerations, it is not surprising to find that the spindle no longer appears in cytological preparations after a critical pressure has been applied before fixation. This is to be interpreted as indicating a liquefaction of pre-existing gel structures, with a consequent loss of molecular organization.

It has been demonstrated that the pattern of the recovery spindle can be very different from that of the original spindle. High hydrostatic pressure seems able to break down the oriented structure of the original spindle so completely that it re-forms *de novo,* and sometimes with a new polarity. In the re-formation of the spindle much the same protoplasmic material may well be used, but the unit molecules or micells are rearranged in a different manner, just as a pile of second-hand bricks might be rearranged to build a new house. This conclusion can probably be accepted as a generalization for it agrees with the findings in *Urechis* eggs which, in their formation of "half spindles," were even more striking (Pease, 1941), with certain other observations on cytoplasmic systems (cf., Pease, 1940), and with general theory.

It is not clear just what does orient the new spindle axis in *Tradescantia* PMC. Cytasters accomplished this end in *Urechis* eggs, and obviously played the important role. These were never observed in the PMC material. Instead, we find a strong tendency for the new axis to coincide more or less with the original. The recovery spindle encountered one unusual difficulty in its organization in that the chromosomes were no longer completely separate entities. After the higher pressures there was usually a continuous plate of fused chromosomes in the equatorial region. Continuous fibers did not, indeed could not, penetrate this obstruction. However, note that homologues were not even found as half spindle components. Continuous fibers were only found sweeping around the blocking mass leaving the core of the spindle devoid of visible oriented structure except for traction fibers. Apparently, therefore, the continuous fibers are entirely a product of the cytoplasm, and are not directly related to the chromosomes. The latter, in fact, are obstacles to be by-passed. This does not, however, preclude the possibility of a generalized interaction between chromosomes and cytoplasm in that the former may "activate" the latter to form gel structures. Such an "activation" was quite definitely shown by *Urechis* eggs recovering from the effects of hydrostatic pressure (Pease, 1941). A more accurate interpretation might be not to stress the continuous fibers as such, but to consider them simply as an index of a more fundamental structural organization of molecules. They thus may signify nothing more than the basic pattern of an extensive gel framework.

On the other hand, the kinetochore apparently quite specifically "organizes" the protoplasm to form the attached traction fiber. This process is partially separable from the development of continuous fibers. We have good reason for believing that developing traction fibers simply follow the path of least resistance in the structural pattern of the bulk of the spindle, which, in turn, is expressed by the distribution of the continuous fibers. Thus the structural pattern of the body of the spindle limits the course taken by the traction fibers as they develop outwards away from the kinetochores. It seems likely that this is a progressive wave of molecular or-

ganization. This view is quite similar to that of Schrader (1932), although based upon different evidence. However, it is fundamentally distinct from that of Belar (1929) who supposed a very different relationship between traction and continuous fiber. Further tentative conclusions on the growth of traction fibers have already been given in describing the experimental results.

The extension of the attachment region in chromosomes subjected to relatively low pressures indicates a real pull by or through the traction fibers. It is almost impossible to imagine that it could be due to "repulsive forces" between the kinetochores for, if that was so, the extension should continue to increase with progressively higher pressures which further soften the chromosomes. Instead, we find the extension to be subnormal while we still have evidence of traction fibers and anaphase movement (at 4,000 lbs. in the first meiotic division). Our conclusion, then, is that the traction fiber is a reasonably stiff gel. No doubt it progressively loses rigidity with increasing pressure, but it has a margin of strength, and there is no important weakness until a pressure threshold is passed. The extension of the attachment stalk is therefore thought due to a pressure effect upon the chromosome itself so that it is softened, and can be unduly pulled out. The subnormal extension at 4,000 lbs. indicates a significant weakness of either the traction fiber or available force. It is interesting for comparison that the centrifuging experiments of Shimamura (1940) with comparable material (*Lilium* PMC) also lead to the conclusion that the traction fiber is a fairly stiff gelled structure. The latter's work seems to the author to be quite conclusive.

Chromosome structure

It seems obvious that some portion of the condensed chromosome tends to be softened, and finally liquefied, by hydrostatic pressures. Since there was no apparent effect upon uncondensed chromosomes, or upon the spirally coiled chromonemata, the portion affected would seem to be the "matrix" (no morphologically separable "sheath" is visible, and presumably more than a sheath would be involved when the attachment region is extended).[2]

A critical analysis of the data, however, discloses some relationships that cannot yet be interpreted with any assurance of certainty. The normal presence of an attachment stalk, and its further extension under relatively low pressures, suggests that the rigidity of the matrix is normally low, but is further reduced by pressure. One might suppose it to be viscous rather than a stiff gel. While the spindle gels are liquefied by moderate pressures, the matrix is not entirely liquefied until pressures of about 15,000 lbs./in.^{2} are applied when the chromosomes so fuse that they lose their visible identity. Thus a structural viscosity appears to persist and withstand very considerable pressures.

It is a fair assumption that the spindle gels obey Marsland's (1939) law, so that their rigidity is reduced 24 per cent by each pressure increment of 1,000 lbs./in.^{2} Liquefaction then occurs at a critical pressure, when gel linkages tend to break more

[2] In the first paper of this series (Pease, 1941) chromosome aggregation was described in *Urechis* eggs subjected to hydrostatic pressure. The cytological appearance suggested that a "sheath" was involved in this fusion rather than the matrix. The *Urechis* chromosomes were so small, though, that the details were not visible. In view of the present work it seems more likely that the matrix as a whole was involved.

rapidly than they can be formed. Whereas we can probably apply Marsland's law to the spindle gels, it does not seem applicable to the chromosome matrix, unless we assume that the matrix material has a much lower pressure/rigidity constant than cytoplasmic or spindle gels, i.e., much less than 24 per cent per 1,000 lbs./in.^{2} That other different gels *in vitro* do, in fact, have different constants has been demonstrated by Marsland and Brown (1942).

There is yet another aspect of chromosome structure to be considered. Why is it that with increasing pressures we find chromosomes rounding up and tending to fuse into a single mass? This looks like an interfacial phenomenon to be explained on the basis of surface tension laws. We do not observe this with uncondensed chromosomes. The author does not see how these and related observations can be explained except by the assumption that a true interface does exist between condensed chromosome and surrounding protoplasm (cf., Hirschler, 1942). Many workers do not believe that there is an osmotically active membrane separating chromosome from protoplasm, although this could explain many of the observations of chromosome swelling. However, a real interfacial boundary would not necessarily imply an osmotically active system.

In any case, it can be presumed safely that any intracellular interface would exert only a very low tension, certainly not more than a fraction of a dyne, or the very few dynes, that have invariably been recorded for water/cell interfaces, or intracellular oil/protoplasm interfaces (cf., Harvey and Shapiro, 1934 and Harvey and Schoepfle, 1939). The presence and properties of dissolved proteins would always prevent high values. Thus any interfacial tension at the surface of a chromosome would be so low that complete rounding of the aspherical shape would occur only when both chromosome and surrounding protoplasm were essentially fluid, and practically without structural viscosity. It is only at a pressure of about 15,000 lbs./in.^{2} that the observed effect indicates these conditions as being nearly fulfilled.

The spindle in chromosome movement

It has already been pointed out that there is a direct and definite correlation between anaphase movement and the presence of a good visible spindle. Hence, our outstanding conclusion is that the presence of gel structure in a spindle is essential for anaphase movement. When the gel rigidity is sufficiently reduced, the movement necessarily ceases. Other types of experimentation have less directly led to the same conclusion (cf., particularly the work of von Möllendorff, 1938 and 1939, on the specific effects of chemical agents). On the other hand, hypotheses involving attractive or repulsive forces are well nigh incompatible with the results. It is hard to imagine hydrostatic pressure affecting such forces, particularly in the low pressure range. Under pressure, with conditions of reduced viscosity, the chromosomes should move apart all the more rapidly and easily if such forces were involved. Furthermore, since Marsland's law relating pressure and viscosity expresses a logarithmic relationship, the effect should be most noticeable in the low pressure range. Obviously this is in direct disagreement with the present findings.

But what is the role of gel structure in anaphase movement? Certainly there are at least two separable structures to be considered—the traction fibers and the spindle mass.

Considering the traction fibers first, Cornman (1944) in a thought-provoking review comes to the conclusion that they are contractile structures and supply the

force for movement. However, Cornman ignores one major difficulty in his otherwise excellent analysis. No one has yet been able to demonstrate that traction fibers thicken as they shorten, although this would be expected if we were dealing with contractile bodies. The author has certainly seen no evidence of this in his own preparations, nor has he been able to observe the converse of any visible thinning when a traction fiber was extended all the way across the cell from one pole to the other. We, therefore, seem to require a different explanation.

It is the author's thought that Schrader (1932) was correct in regarding traction fibers as being no more than passively semi-elastic structures. This has been given excellent experimental foundation by Ris (1943) who has been able to measure directly anaphase movement in living cells (insect spermatogonia and spermatocytes). In some cases he has demonstrated that anaphase movement is very definitely a two step process. The first, relatively rapid movement can be explained as due to the release of elastic tension so that the traction fibers do actually shorten. The remaining movement is then due to the spindle mass, with the traction fibers serving simply as passive connections to the chromosomes. Lewis (1939) produced an accelerated motion picture of dividing fibroblasts *in vitro* which beautifully showed the same phenomenon, although he has not commented upon it.

A general hypothesis of anaphase movement can be advanced on the assumption that the traction fiber is anchored at one end to the chromosome, and along some of its length to the larger gelled mass of the spindle which, in turn, is in motion. Thus it is simply a more or less elastic connection from the spindle body to the chromosome—a rope, so to say, between the machine and the load. This interpretation forces our attention to the body of the spindle.

The analysis of anaphase movement by Belar (1929) does much to delimit the problem, even though we cannot accept his general hypothesis. He demonstrated that it was impossible to account for the total movement on the basis of simple swelling or elongation of the main spindle mass (or, more specifically, the Stemmkörper). There is, however, an obvious way to avoid the difficulties outlined by Belar (other than his own solution), and still be consistent with his findings and other knowledge.

It is proposed that motion and force may be imparted to the spindle mass by means of two phase transformations. The postulate supposes that gel material is added either in the interzonal region [3] or along the greater part of the spindle, while a proportional solation occurs at the poles. Thus a material circulation is established, but a circulation by means of sol-gel-sol transformations rather than within a single phase. Actually a somewhat comparable idea has been proposed by Wassermann (1929 and 1939). Such an idea would be regarded by many as entirely too speculative, and not subject to either proof or disproof. The author, however, wishes to point out some comparable effects which are not likely to be known to most cytologists.

Dan *et al.* (1938 and 1940) discovered a remarkable phenomenon in dividing sea urchin eggs. After the furrow completes its intrusion, an entirely new region of gelled cortex is added in the center of the furrow region as the original cortical

[3] Note that Schmidt (1939) did not find birefringence with polarized light in the mid-region of sea urchin egg spindles, and that Shimamura (1940) found this to be the "weak" region in centrifuging experiments upon *Lilium* PMC.

material backs out. Pease (1943) calculated that this *de novo* cortex came to cover about 11 per cent of the cell surface. This gel growth is obviously analogous to a system that could very well work within a spindle.

Since the advent of hydrostatic pressure techniques, it has also become clear that all sorts of other cell processes involving movement are dependent upon gel structure. Thus amoeboid movement, cyclosis, streaming, cytoplasmic division, the movement of pigment granules, and the pole cell nuclei of *Drosophila* eggs, and even sperm penetration both through the egg surface and also to their final central position all cease (reversibly) when the gel is liquefied. All of these movements depend upon the rather unexpected, and admittedly little understood, properties of protoplasmic gels. Obviously the gel rearranges itself, and is itself in motion (cf., the review of Marsland, 1942). No doubt gel-sol transformations are usually if not always involved along with the rearrangement. Thus we do find empirically a common denominator for all movements other than such specialized activities as muscle contraction and ciliary motion. The author believes that a general theory of anaphase movement is in sight, and that it will come from a better physicochemical understanding of protoplasmic gel-sol systems.

Summary

Hydrostatic pressures have been applied to *Tradescantia* pollen mother cells as a technique for studying the structure of division spindles and chromosomes and the mechanics of anaphase movement. The procedure has given pertinent information by virtue of the fact that increasing pressures progressively reduce gel rigidity. Sufficiently high pressure results in liquefaction. Yet the effects are reversible.

The spindle of the first meiotic division was but slightly affected by 4,000 lbs./in.^{2} pressure, yet was mostly liquefied by 5,000 lbs. The spindle of the second meiotic division withstood about 2,000 lbs. more pressure. The somatic cells were even more resistant.

Condensed chromosomes were significantly softened by even 1,000 lbs./in.^{2} pressure as indicated by an undue elongation of the kinetochore stalk. Fusion bridges became particularly obvious when 3,000 lbs. was applied. Significant shortening and rounding occurred at 4,000 lbs. Total fusion and rounding, indicating complete liquefaction of the matrix, did not occur until pressures of 15,000 lbs./in.^{2} were applied. The fusion and rounding appeared to be a surface tension effect, and suggested the existence of a true interfacial membrane between condensed chromosome and cytoplasm. Not even these highest pressures, however, affected the uncondensed prophase chromosomes so that the effect of pressure was thought to be only upon the matrix material.

Chromosome movement was limited to those pressures which did not liquefy the spindle. The presence of fusion bridges, however, resulted in very abnormal movement.

After the release of high pressures, spindles re-formed. That these were *de novo* structures was indicated by their sometimes abnormal orientation, by the frequency of multipolar spindles, and by abnormalities in the course of traction fibers. Thus, the traction fibers of two homologous chromosomes might go to a single pole. Abnormalities made it seem likely that the growth of traction fibers was in a large measure independent of the growth of the body of the spindle. The direc-

tion of growth of the traction fiber was not specifically oriented until it reached the oriented bulk of the spindle.

Chromosome movement in recovery material was abnormal in that the fusion bridges persisted. Thus the chromosome matrix which had been liquefied, had become highly viscous once more. Under such circumstances, homologous chromosomes frequently went to a single pole, and the traction fiber to the other pole extended all the way across the cell. However, such traction fibers were not thinner than normal.

The outstanding conclusion is that a gel structure in the spindle is essential for anaphase movement. The traction fiber apparently serves as nothing more than a semi-elastic connection between the chromosome and the main mass of the spindle which, in turn, is in motion. It is suggested that motion and force is imparted by means of sol-gel-sol transformations, with gel being added to the central bulk of the spindle while a proportional solation goes on at the poles.

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